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(54) Title: METHOD FOR ACCELERATING GROWTH RATES

(57) Abstract

Upon subjection to a low energy electrical field of varying and critical frequencies and low field strengths the growth rate of an organism disposed in a growth medium exhibits a significant increase over power-off controls.

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METHOD FOR ACCELERATING GROWTH RATES

1. Field of the Invention

This invention relates to a method for increasing the growth rate of biosystems. More particularly, this invention provides a method for accelerating the growth rates of organisms which involves the use of low energy electrical fields produced by a unique combination of applied low field strengths and extra-low electromagnetic frequencies.

2. Prior Art

The effects of electrical fields on the stimulation of growth and regeneration in various biosystems have been studied. Mcelhaney et al., J. Biomech. 1:47 (1968) disclose the use of electrical fields to stimulate bone formation. The use of applied magnetic fields to accelerate growth in microorganisms is shown in U. S. Patent 3,871,961. Sewage decomposition promoting microorganisms are stated in U. S. Patent 3,336,220 to exhibit increased activity after an alternating electric current was passed through the sewage. MacKenzie in New Scientist, 28 January 1982, pp. 217-220 reports on electrical fields which develop in connection with loss of limbs and which are implicated in the resultant regeneration phenomena. The effects of electrical fields on movement in an amoeba was reported in Science, 187:357 (1975).

Much of the work performed studying the effects of electrical fields on microorganisms has involved the study of mutagenesis and also sterilization, e.g., see Coate, W. B. et al. 1970, "Project Biological Effects Test Program Pilot Studies Final Report." Hazilton Laboratories, Inc., available from NTIS as AD717409, U. S. Patent 3,876,373 and Food Tehnol, 8:361 (1954).

It is an object of this invention to provide a unique and practical methodology utilizing low energy electrical fields to accelerate growth rates when applied to a living biosystem. It is a further object of this invention to increase such growth rates without producing mutants and without a measurable increase in the temperature of the biosystem. It is a still further object of this invention to provide an energy efficient, low cost and effective process for significantly reducing the growth cycle time of a biosystem.

DESCRIPTION OF THE INVENTION

These and the other objects of this invention are accomplished by a process for accelerating the growth rate of a live organism which comprises disposing the organism in a suitable growth or nutrient medium and subjecting the organism to an alternating electrical field at an electrical field strength and frequency combination which causes the growth rate of the organism to increase over the growth rate of the organism when not subjected to the alternating electrical field. The desired increase in growth rate is independent of current density and is exhibited at varying frequencies and electrical field strengths. However, as far as has been tested there appear to exist certain critical frequencies, or regions of frequencies, at which accelerated growth occurs and outside of which there is minimal or no effect. Also at such effective frequencies there exists a threshold electrical field strength below which there is no effect. It has also been found that, within the ranges of effective frequencies and field strengths, the resultant electrical field is a low energy field which does not produce mutants of the organism; nor is there a measurable increase in the temperature of the biosystem. Furthermore, in the preferred ranges of effective frequencies and strengths there is no electrolysis of the growth medium. It has

found that the effective frequencies and voltages are independent of current density. However, there is a practical limitation on current density in that one would not employ currents that adversely affect the biosystem. Tests have been conducted at less than 0.1 mA (milliamp) and it is believed that absolutely no current is required to effectively increase growth rates, so long as effective field strengths and frequencies are utilized in carrying out the invention.

Definition of Terms

"Growth Rate (rate)" - This is obtained by taking the Klett units over time and calculating the doubling time for the culture. This is done by plotting the Klett unit number of each reading on the log scale of a semi-log graph (wherein the y axis is the semi-log scale and the x axis is time). The slope (m) of the curve is then calculated using the following formula:

$$m = \frac{E_{xy} - \frac{E_x E_y}{N}}{E_x 2 - \frac{(Ex)^2}{N}}$$

wherein m is the slope, x is time, y is the semi-log, and N is the number of individual Klett readings.

"Klett units" - a unit of measurement of optical density employing a Klett-Summerson photoelectric colorimeter.

"LeeGs value (L)" is obtained by dividing the growth rate of the test sample by the growth rate of the control sample thus

$$L = \frac{G \text{ test}}{G \text{ cont}}$$

"Correlation (coor)" is calculated in accordance with following mathematical sequence:

$$corr = R = \frac{Ms_x}{s_y}$$

wherein s is the standard deviation of the x and y axes of the semi-log graph referred to in the definition of growth rate.

"Dev" is an abbreviation for the standard deviation between the control groups calculated in the following manner:

$$Dev = \left(\frac{sum(x^2) - \frac{[sum\ (x)]^2}{N}}{N - 1} \right)^{1/2}$$

wherein x is the value of the growth rate of each control group and n is the number of control groups.

"L-broth" - a growth medium consisting of 0.8% NaCl, 0.8% glucose and 0.7% bacto-tryptone, all percentages being weight to volume of tap water.

"Biosystem" - a system comprised of the organism disposed in a medium suitable for its normal growth.

"Hz" - abbreviation for hertz, a unit synonomous for cycle per second.

"KHz" - 1 kilohertz or 1000 hertz.

"ATCC" - American Type Culture Collection a depository for microorganisms located in Rockville, Maryland.

Example 1

An overnight exponential culture of prototropic E.

coli K-12 (Yale University depository number CGSC No. 4401) growing at 37°C, in L-broth was diluted with thermally equilibrated and sterilized L-broth to a density of 1 x 10^6 cells per milliliter. Portions of this culture were then randomly placed into 10 ml. glass test tubes made of the type glass specified for the colorimeter employed. Each test tube contained two platinum electrodes, spaced 4 centimeters apart, wired in series to a variable frequency generator and in parallel to an oscilliscope, and equipped with means for oxygen input to the growth medium. The tubes containing the fractionated culture medium were maintained at constant temperature in a thermostatically controlled water bath and each subjected to an electromotive (EmF) field. Temperature was monitored with a glass/mercury thermometer. Each tube was isolated from the field of the other tubes by brass screening. This brass screening was grounded to totally eliminate any electrical transfer from one tube to the other. The culture densities of each test tube were tested with a Klett-Summerson photoelectric colorimeter by measuring, at 20-30 minute intervals over a 3 hour period, increasing optical absorbences at 660 nanometers. Controls were also run using cells that were inactivated by subjecting them momentarily to 60°C and quick cooling to 37°C. Each set of controls consisted of three test tubes which were measured at 10, 100 and 1000 hertz to establish the background of optical absorbence effects due to the electroplating of cells and growth medium components onto the electrodes and also to ascertain any electrolytic effects that might be present. The EmF fields were produced by the variable frequency generator and monitored via the oscilliscope. The low energy fields were applied by

passage of alternating current through the growth medium between the platinum electrodes.

The results obtained with E. coli, following the above procedure are set forth in the following tables.

Table 1

At 37°C, 1KHz, 10V.cm

time	0	20	40	60	80	rate	coor
con	37	39	42	48	52	0.37	0.99
exp	37	41	45	58	53	0.47	0.92

1.27

****

Table 2

At 34°C, 1KHz, 18V/cm

time	0	30	60	rate	coor
con1	66	68	72	0.12	0.98
con2	50	52	55	0.14	0.99
exp1	49	53	58	0.24	1.00
exp2	93	98	105	0.17	1.00

cont avg. = 0.13, dev = 0.01
L1 - 1.85, L2 - 1.31

****

Table 3

At 37°C, 10KHz, 5V/cm

time	0	30	60	rate	coor
con1	65	76	88	0.44	1.00
con2	65	78	89	0.46	1.00
exp1	71	84	99	0.48	1.00
exp2	66	81	98	0.57	1.00

cont avg. = 0.45, dev = 0.01
L1 - 1.07, L2 - 1.27

****

Table 4

At 37°C, power off

	time	60	90	rate	coor
	con1	88	107	0.56	1.00
5	con2	89	108	0.56	1.00
	exp1	99	112	0.36	1.00
	exp2	98	112	0.39	1.00

cont avg. = 0.56, dev = 0.00
L1 - 0.64, L2 - 0.70

****

10 Table 5

At 37°C, 1KHz, 5V/cm

	time	120	150	rate	coor
	con1	141	154	0.25	1.00
	con2	130	148	0.37	1.00
15	exp1	137	151	0.28	1.00
	exp2	135	151	0.32	1.00

cont avg. = 0.31, dev = 0.08
L1 - 0.90, L2 - 1.03

****

Table 6

20 At 37°C, 1KHz, 5V/cm

	time	0	10	95	110	rate	coor
	con1	62	64	91	94	0.34	1.00
	con2	58	61	89	95	0.42	1.00
	exp1	77	82	123	117	0.79	0.98
25	exp2	64	68	94	95	0.65	0.99

cont avg. = 0.38, dev = 0.06
L1 - 2.08, L2 - 1.71

****

Table 7

At 36°C, 100Hz, 5V/cm

time	0	30	60	rate	coor
con1	133	138	153	0.20	0.96
con2	140	144	152	0.12	0.98
exp1	135	139	152	0.17	0.96
exp2	144	149	159	0.14	0.98

cont avg. = 0.16, dev = 0.06
L1 - 1.06, L2 - 0.88

****

Table 8

At 37°C, 1KHz, 10V/cm

time	0	30	60	90	120	rate	coor
con1	120	135	153	174	200	0.36	1.00
con2	132	141	158	179	200	0.30	1.00
exp1	128	141	164	185	200	0.34	1.00
exp2	125	137	160	180	200	0.35	1.00

cont avg. = 0.33, dev = 0.04
L1 - 1.03, L2 - 1.06

****

Table 9

At 34°C, 500Hz, 5V/cm

time	0	30	60	rate	coor
con1	132	144	158	0.26	1.00
con2	129	145	155	0.27	0.99
exp1	121	136	146	0.27	0.99
exp2	112	120	133	0.25	0.99

cont avg. = 0.27, dev = 0.01
L1 = 1.00, L2 = 0.93

****

Table 10

At 40°C, 5KHz, 5V/cm

time	0	30	90	rate	coor
con1	108	119	142	0.26	1.00
con2	107	113	139	0.25	0.99
exp1	107	121	140	0.26	0.99
exp2	113	126	142	0.22	0.99

cont avg. = 0.26, dev = 0.01
L1 = 1.00, L2 = 0.85

****

Table 11

At 37°C, 1KHz, 8V/cm

time	0	30	60	90	120	rate	coor
con1	98	103	106	115	122	0.16	0.99
con2	103	105	109	116	125	0.14	0.97
exp1	95	100	109	120	122	0.20	0.98
exp2	95	102	115	118	120	0.18	0.95

cont avg. = 0.15, dev = 0.01
L1 = 1.33, L2 = 1.20

****

Table 12

At 37°C, 100Hz, 5V/cm

time	0	70	90	rate	coor
con1	107	127	129	0.19	0.99
con2	93	111	115	0.21	1.00
expt	95	116	113	0.19	0.94

cont avg. = 0.20, dev = 0.01, L = 0.95

****

Table 13

At 37°C, 1KHz, 3V/cm

time	0	70	90	rate	coor
con1	107	127	129	0.19	0.99
con2	93	111	115	0.21	1.00
expt	84	100	107	0.23	1.00

cont avg. = 0.20, dev = 0.01, L = 1.15

****

Table 14

At 37°C, 10KHz, 5V/cm

time	0	30	60	rate	coor
con1	33	34	37	0.16	0.96
con2	33	34	37	0.16	0.96
expt	33	34	39	0.23	0.94

cont avg. = 0.16, dev = 0.00, L = 1.44

****

Table 15

At 37°C, 1KHz, 4V/cm

time	0	30	60	rate	coor
con1	33	34	37	0.16	0.96
con2	33	34	37	0.16	0.96
expt	38	42	48	0.33	1.00

cont avg. = 0.16, dev = 0.00, L = 2.06

****

Table 16

At 35°C, 1KHZ, 4V/cm

time	0	30	60	90	120	rate	coor
con1	36	41	44	48	54	0.28	1.00
con2	39	44	50	62	79	0.48	0.99
expt	39	45	59	78	103	0.67	0.99

cont avg. = 0.38, dev = 0.14, L = 1.76

****

Table 17

At 34°C, 1KHz, 2V/cm

time	0	30	60	90	120	rate	coor
con1	36	41	44	48	54	0.28	1.00
con2	39	44	50	62	79	0.48	0.99
expt	44	47	53	60	65	0.29	1.00

cont avg. = 0.38, dev = 0.14, L = 0.76

****

Table 18

At 37°C, .0Hz, 4V/cm

time	0	30	60	85	rate	coor
con1	42	43	44	42	0.01	0.18
con2	37	38	39	34	-0.07	-0.46
expt	35	48	55	70	0.70	0.99

****

Table 19

1KHz, 1V, 0.4% NaCl

time	0	46	61	75	rate	coor
cont	23	31	34	36	0.49	0.98
expt	23	31	36	42	0.66	0.99

(Power was inadvertently on on the control at 1KHz., 0.5V until the time of the 46 minute reading, therefore the valid reading would start at that time. Starting

SUBSTITUTE SHEET

from 46 minutes the results turn out that the growth rate of the control turns out to be 0.45 gen/hr with a correlation of 0.99 and the rate of the experimental is 0.90 gen/hr with 1.00 correlation. This gives L=2.00 which corresponds with previous data.)

****

Table 20

Testing of 0.4 & 0.2% NaCl to show that the effect is not due to current.

1KHz., 1V

time	0	30	60	70	80	90	rate	coor
0.4%	20.0	22.0	25.0	27.5	29.5	32.0	0.43	0.98
0.2%	9.0	10.0	10.0	11.5	12.5	14.0	0.36	0.91

power off

time	0	30	60	rate	coor
0.4%	32.0	35.5	39.5	0.29	0.90
0.2%	14.0	15.0	17.0	0.28	0.99

1KHz., 1V

time	0	10	20	30	rate	coor
0.4%	39.5	42.5	47.5	49.0	0.66	0.98
0.2%	17.0	21.0	22.5	24.5	1.07	0.96

0.4% rate avg. = 0.54, div = 0.16, L = 1.86
0.2% rate avg. = 0.72, div, div = 0.50, L = 2.57

That mutagenesis was not implicated in the acceleration of growth rates for E. coli was demonstrated by presence of carbon dioxide evolution when a culture medium from Example 1 was added to a test tube containing lactose and mineral salts at the temperature of 43°c (Eijkmann-Durham Test for contaminants).

Example 2

The procedure of Example 1 was followed except that the microorganism was Bacillus subtilus (ATCC 6051). The following results were obtained:

power off (control) temp. = 22c

time	0	30	60	rate	coor
A	0.126	0.132	0.145	0.200	0.980
B	0.028	0.033	0.036	0.370	0.980

power 1KHz, 1V/cm

time	0	30	60	90	rate	coor
A	0.145	0.162	0.182	0.206	0.336	1.000
B	0.036	0.041	0.053	0.064	0.553	0.993

A L = 1.68; B L = 1.50 L = 1.59

Example 3

The procedure of Example 1 was employed except that 2% Bacto yeast extract (Fleichman's fresh active yeast) was employed in place of E. coli and was grown at 25°C in an aqueous solution of 0.3% Bacto-Tryptone and 4.0% glucose (percentages are weight to volume water). The following results were obtained:

power off (control)

time	0	60	120	rate	coor
1	67	69	71.5	0.05	1.00
2	99	100	105	0.04	0.93

1KHz., 1V/cm

time	0	60	120	180	rate	coor
1	71.5	75	81	83	0.08	0.98
2	105	110	113	118	0.05	1.00

power off (control)

time	0	60	rate	coor
ch-1	83	85	0.03	1.00
ch-2	118	120	0.02	0.99

power 100 Hz., 1V/cm.

time	0	60	100	rate	coor
1	85	87.5	89	0.04	1.00
2	120	123	124	0.03	0.99

cont ch-1 avg = 0.04, dev = 0.01
cont ch-2 avg = 0.03, dev = 0.01
rate ch-1 1KHz. = 0.08, L = 2.00

rate ch-2 1KHz. = 0.05, L = 1.67
L 1KHz. avg = 1.84
rate ch-1 100 Hz. = 0.04, L = 1.00
rate ch-2 100 Hz. = 0.03, L = 1.00

Example 4

This example demonstrates the efficacy of the process of the invention in accelerating plant growth rates.

Glass or plastic containers containing soil and bean seeds (Bountiful Green Bush variety of Pinto) were configured for generation of low energy electrical fields in the following four modes:

1. parallel brass screens above and below the soil between which the beans are positioned without touching the screens

2. one brass screen parallel to the side of the container with the seeds spaced apart therefrom with intervening soil in between the seeds and the screen

3. parallel wires in the horizontal plane of the bean seeds and spaced apart therefrom

4. one brass screen at the bottom of the container below the bean seeds.

Each container had from 4-8 bean seeds planted therein. All wires and screens were connected in series to an alternating current generator capable of producing multiple functions (e.g., sine or square waves). For each planted container connected to the generator there was a power-off control container of the same configuration. All other conditions were the same for controls and test groups. To each test container were applied 22 volts (approximately 2.5 volts/cm.) at 1 KHz over a period of one week. At the end of the test period the test groups averaged about 8.5 inches in height, whereas the controls had just commenced to germinate. The test group had significantly higher germination success than the controls. At comparable stages of development the test group was more vigorous in appearance than the controls and hence appeared to exhibit an increase in developmental success.

Example 5

Into a glass container was added a brass screen upon which was placed fruit fly medium containing freshly laid eggs. On top of the medium was placed another screen which along with the screen on the bottom of the container, was connected in series with a variable frequency generator. To the test sample was applied a field of 1KHz, 22V (about 2.5 V/cm.); the control was a power off sample. The flies (Drosophila melanogaster) were then observed to eclosure (reaching of adulthood), and observed to show an earlier eclosure in the test sample than the control. The test sample also exhibited a greater hatching and eclosure success

and also was more vigorous (i.e., observably significantly more active than the control.)

The foregoing examples demonstrate that 1000 Hz and 10,000 Hz are highly preferred effective frequencies for growth rate acceleration with widely differing species of organisms at field strengths, which can be expressed in terms of volts per centimeter, ranging upwards of from about 1 volt per centimeter to about 22 volts per centimeter at the expressed frequencies. However, positive growth rate acceleration can be achieved at selected frequencies ranging upwards from 10 Hz to about one megahertz (MH_z), the only practical limitation being that point at which mutagenesis occurs. A wide range of field strengths can be employed at each selected effective frequency with a concomitant threshold field strength below which no effect occurs. Again practicality dictates that the upper limit for such field strengths is that point at which a measurable temperature increase occurs in the biosystem. Selection of these critical frequencies and field strengths can routinely be accomplished by performing a simple series of tests as follows:

> Set up electrodes so that electrical field is applied across the biosystem, to which electrodes are applied selected frequencies at a set minimal field strength of 1V/cm. Upon discovery of an effective frequency for growth rate increase, various field strengths are applied at the set effective frequency to determine the effective field strength

range. With microorganisms it is recommended that the system be designed essentially as described in Example 1. With plant or animal species any one of the systems described in Example 4 can be utilized. If no effective frequency is found at 1 volt/cm., repeat procedure at 5 volts/cm. and at 5 volt increments thereafter until an effective growth rate increasing frequency is found or until a measureable temperature increase occurs in the biosystem using a standard glass mercury thermometer.

Increase of growth rates while improving developmental success and vitality of some organisms in accordance with this invention has important commercial implications for industrial fermentation, as well as, agricultural and pharmaceutical production industries. Improved yields and increased production capacities are direct advantages. Shorter organism growth cycle could open new vistas in diagnostic medicine as well as other areas of medicine such as bone marrow regeneration, organ regeneration, tissue revitilization and the like.

What is claimed is:

1. A method of increasing the growth rate of a living organism which comprises disposing the organism in a growth medium and subjecting the organism to a low energy electrical field produced by a combined field strength and frequency effective for increasing the growth rate of said organism.

2. The method of claim 1 wherein said field strength is above about 1 volt per centimeter and said frequency is selected from about 10 to about 10^6 Hz.

3. The method of claim 1 wherein said organism is a microorganism or plant or animal.

4. The method of claim 3 wherein said voltage is above about 1 volt per centimeter and said frequency is selected from about 1000 Hz and about 10,000 Hz and the resulting electrical field does not increase the temperature of the medium.

5. The method of claim 3 wherein said voltage is selected from above about 1 volt per centimeter to about 22 volts per centimeter and said frequency is about 1000 Hz or about 10,000 Hz.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02271

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12N 13/00;A01G7/04;A01K 67/00
U.S. Cl: 435/173; 426/2; 47/1.3; 119/1

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S.	435/173,243;426/2;47/1.3;119/1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X Y	US, A, 3,336,220 (NEIDL) 15 August 1967, See col. 2, lines 15-20, col. 3, lines 5-20 and 45-50, and col. 6, lines 27-30.	1,3 2,4,5
X Y	EP, A 0041373 (WORTH) 12 September 1981, see entire document.	1,3 2,4,5
X Y	SU, A, 721 480, 18 March 1980, see translated abstract.	1,3, 2,4,5
X Y	SU, A, 842105, 30 June 1981, see translated abstract.	1,3 2,4,5

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
12 January 1987	14 JAN 1987
International Searching Authority 1	Signature of Authorized Officer 20
ISA/US	David M. Naff

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X/Y	DE, A, 2841455 (GERTH) 10 April 1980, see translated abstract.	1,3/2,4,5
A	SU, A, 745939, 17 July 1980, see translated abstract.	
A	US, A, 2,492128 (SHROPSHIRE), 20 December 1949, see entire document.	

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	US, A, 3,871,961 (GIANESSI), 18 March 1975, see entire document.	
A	US, A, 4,487,834 (BRIGHTON), 11 December 1984, see entire document.	